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Abstract: *Abstract: We propose a modelling strategy to determine from a large set of reactions the smallest set necessary to be compatible with a compendium of required properties and experimental data. We applied this strategy to define a model of hepatic fatty acid metabolism integrating both metabolic pathways and the contribution of some major genetic regulators. We considered the fatty acid metabolism in hepatic cells of mice during a 72 hours fed-to-fast transition and we tested the key role of oxidation and elongation in fatty acid metabolism*

Keywords: Network modelling, differential model, fatty acid metabolism, abstraction.

1 Introduction

Fatty acid metabolism plays a central role in energy homeostasis and related disorders such as the metabolic syndrome, obesity or type-II diabetes. In farm animals, the distribution and composition of body lipids is of great importance for meat quality.

Synthesis, degradation, and transformation of fatty acids in cells are performed by more than 300 well-known enzymatic reactions [6] participating to different processes called *pathways* (e.g., glycolysis, fatty acid oxidation, ...) [6,4]. These reactions are regulated at the metabolic and genetic levels by hormones and nutrients. To describe these biochemical transformations and their genetic regulation, abundant knowledge exists for different cells types and organisms (221751 references for *fatty acid metabolism* in Pubmed; 1742 for *PPAR*α), bu the simple aggregation of knowledge cannot explain a global phenomenon caused by interations of regulations. While the metabolic pathways involved in fatty acid homeostasis are quite well understood and referenced, the genetic regulations are much less accurately described.

To date, several biochemical models that describe extensively a single pathway, such as glycolysis [5,12] have been proposed. A major disadvantage of full-scale models lies in the large number of parameters required compared to the limited information available from experiments. Model-reduction

techniques for complex chemical kinetics pool variables have been proposed, based on timescales or correlation arguments [2,7]; they are however dependent on the availability of experimental data and kinetic coefficients. Other models have considered the genetic regulation of few enzymatic reactions [11]. To the best of our knowledge, there is no model for the complete fatty acid metabolism along with its regulations. The purpose of this study is to build a reduced model, aiming at identifying and quantifying the key regulators of fatty acid metabolism in the mouse liver, during a fed-to-fast transition.

In this study, we focused on liver because this is the main synthesis organ allowing the conversion of excess dietary carbohydrates into triglycerides and also an important catabolizing organ where fatty acids are oxidized and transformed into other products such as ketone bodies[4].

We propose to build a *reduced dynamical model* in which single reactions are grouped as much as possible into a limited number of integrated pathways. First, a complex knowledge model is obtained from the current state of the art. Then, a reduction strategy is applied, based on a *compendium of required properties* provided by experts. This compendium can be seen as the degree of detail that is considered as essential in developing the model; the more accurate it is, the more complex the model will be. The knowledge model is simplified by merging molecules or reactions into pathways and aliasing regulators that do not need to be represented explicitly given the compendium. Lastly, we use an experiment to decide if the degree of detail has been correctly assessed. This will be illustrated by the analysis of the synthetic and oxidation pathways in the context of wild-type and PPARα-/- mutant mice.

2 Model abstraction

We inted to build the smallest dynamical model described by differential equations representing fatty acid metabolism, while being consistent with experimental data and biological knowledge. In this framework, the *biochemical kinetic model* is defined as a differential equation model where the time variations of components are governed by reactions, influx and efflux, all being regulated genetically or metabolically. Together with the model, we considered a *compendium of required biological properties* and experimental data. The model was considered *consistent* if the required behaviour can be predicted by the model, and if the model fits with experimental data with reasonable precision.

We first built a model from a literature study after a discussion with experts. Then, the reduction of each element of the model was considered: (a) A *reaction* R was removed from the model if the compendium of required properties was still satisfied when the reaction rate is fixed to zero. Biologically, this means that R was not necessary to influence the process from a phenomenological point of view. (b) A *regulation process* (i.e., the dependency of a reaction R on a variable X), could be removed if the biological properties were still satisfied when the variable X was considered as a constant in the expression of R. (c) A *set of variables* could be eliminated when they are *intermediate components* of a pathway, meaning that they do not have an influence outside the pathway. Then, reactions in the pathway could be reduced as a single reaction regulated by the regulator of its rate-limiting step. (d) Two *variables were pooled* if they participated in the same pathway with the same kinetic coefficients, or when they were correlated regulators. For example, glucose can be pooled with insulin because of their synergistic functional effects; even though their effects have been proved to be independent.

The model was said to be *minimal* if no reduction could be performed without losing consistency. The model was qualitatively verified by confronting model predictions to biological behavior (e.g., lipogenesis during the fed state, lipolysis and fatty acid oxidation in the fasting state). With this

approach, the resulting variables then represent *synthesized components*, which were single molecules or groups of molecules that do not need to be distinguished for a given set of observed properties. Therefore, the main mechanisms included in the model were not *a priori* selected from knowledge, but because they were needed to respect biological behaviour.

3 Application : a minimal model for fatty acids metabolism in mouse liver during a fed-to-fast transition

State of the art on fatty acid metabolism and its regulation The following pathways were chosen from metabolic databases [6] and litterature reviews [4] as related to fatty acids (FA) metabolism : glycolysis, tricarboxylic acid (TCA) cycle, ketogenesis, the pentose phosphate cycle, FA oxidation, FA synthesis, long chain polyunsaturated fatty acids (LCPUFA)synthesis. We obtained a model with 61 metabolites.

Despite being a multi step enzymatic process, de novo FA synthesis from acetyl-CoA in eukaryotes is accomplished through the catalytic activities of only two enzymes (gene products): acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). Among the several transcriptional regulators involved in the balance between FA synthesis and oxidation, two classes of central regulators emerge: liver X receptors (LXR) that act in synergy with sterol regulatory element binding protein (SREBP1) in promoting hepatic lipogenesis [1], and peroxisome proliferator-activated receptor (PPARα) controling the expression of numerous genes involved in FA uptake and β-oxidation [10].

Compendium of required biological properties and experimental data The degree of detail of the model was determined to include the main pathways dependencies described in the literature (condition 1), fundamental behavior related to the context of the fed-to-fasting transition (conditions $2-6$) and experimental data related to a fasting protocol [3] (condition 7).

1. **Pathway dependencies** The cell needs energy, therefore it uses ATP. In the liver, ATP can be produced from glucose or from FAs. The liver can synthesize lipids from glucose. This synthesis is active only when the glucose concentration exceeds the minimum concentration needed to cover energy needs by prod ucing ATP in the TCA cycle [4]. We consider that ketogenesis cannot happen without FA oxidation. Since the liver is aerobic, the TCA cycle is running at a high speed. FA oxidation and synthesis cannot occur at the same time. Acetyl-CoA does not accumulate into the cells. FA can be imported or exported from the liver. Enzymes are synthesized and degraded.s
2. **In the fed state**, (a) FA oxidative reactions are null; (b) ketogenesis is also absent; (c) enzymes of FA oxidation and ketogenesis are not synthesized; (d) FA synthesis is active; (e) the corresponding enzymes and LXR-SREBP1 are synthesized.
3. **During fasting**, (a) there is no FA synthesis; (b) enzymes of FA synthesis are not synthesized.
4. **In wild-type mice, during fasting**, (a) transcriptional genetic activators (GA) of FAs oxidation are synthetized; (b) Ketogenesis is active; (c) Oxidative enzymes are synthetized; (d) Blood ketone concentration is elevated.
5. **In wild-type mice**, Ketogenesis is active only when oxidation is high.
6. **When PPARα is genetically lacking** (e.g. in PPAR$alpha$ knock-out (PPAR-/-) mice), (a) ketogenesis and (b) oxidation are low, and not regulated during fasting.
7. **Wild-type and PPAR-/- experimental data**. Additionnally, we expect our model to be consistent with experimental data obtained on wild-type and PPAR-/- mice during a 72 hour period of fasting. Mice were sacrificed after different periods of fasting (0, 3, 6, 9, 12, 18, 24, 36, 48, 60, 72

hours) weighed and lipid hepatic mass was determined. Blood was collected, and glucose concentrations were measured in the plasma. Liver and epididymal white adipose tissue were dissected and weighed. Lipids were extracted from liver and adipose tissue, and FAs (CC12:0, C14:0, C16:0, C16:1ω9, C16:1ω7, C18:0, C18:1ω9, C18:1ω7, C18:2ω6, C18:3ω3, C20:1ω9, C20:3ω6, C20:4ω6, C20:5ω3, C22:6ω3) composition was determined in the tissues by gas chromatography.

Reduction hypotheses for mouse hepatic fatty acid metabolism during fasting In the biological context considered, we performed a first reduction of components and reactions: (a) Lactate production by liver is not considered since liver metabolism is aerobic. (b) NADH and FADH2 were abstracted as intermediates involved in ATP production, and we considered these as being oxidized in the respiratory chain providing respectively 3 and 2 ATP. (c) In liver, because glucose transport is important, we assumed that intracellular glucose concentration was similar to blood glucose concentration. Therefore, glycogenolysis and neoglucogenesis are not needed in the model. (d) The timescale of regulation of pentose phosphate cycle is short compared to the integration time step of the model (3 hours); pentose phosphate pathway was reduced to the consumption of glucose and ATP instead of NADPH consumption. (e) The consumption of ATP by the remaining (and not modelled) part of cell metabolism is reduced to a single function. (f) Two components were included in the model for the transcriptional control of FA oxidation and synthesis (denoted by Activating Transcription Factors). They relate to LXR-SREBP1 and PPARα, respectively, or may be a combination of several transcriptional regulators involved in FA oxidation or lipogenesis. (g) The synthesis of glycolysis, ketonegesis and TCA Cycle enzymes are modelled by a single reaction for each pathway. (h) A unique reaction stands for β-oxidation of each FA. (i) At this stage, the model does not need to include transformations from one FA to an other because there is no biological behaviour concerning a specific FA, they are all merged into a single node FA.

From the knowledge model depicted in Fig 1, we derived a mathematical model by using Michaelis-Menten regulated functions for the reaction rates. The model was consistent with the desired biological behavior, but was not able to fit properly individual fatty acid behaviour in wild-type experimental data.

Challenging the model with experimental data The remaining question is whether the abstraction of the model is sufficient to match data available on fasting PPAR-/- and wild-type mice. During fasting, active pathways are lipid influx, oxidation, desaturation and ketogenesis. The other pathways are not active, hence no information about these can be deduced from the experiment.

In PPAR-/- mice, oxidation is low and a massive accumulation of the total amount of FAs in the liver is observed. The simplest model includes only FAs influx with no transformation between FAs : the FA liver influx is assumed to be proportional to adipose tissue efflux. This corresponds to a dynamical model $\frac{dF_{Liver}}{dt} = Imp_F = -K_{imp}\frac{dF_{AdiposeTissue}}{dt}$ for each fatty acid F, the import ratio K_{imp} being the same for all FAs. We found $K_{imp} = 0.22$ with a fitting procedure on the sum of all FAs. A statistical score taking into account the variability of observations and predictions showed that this import model is accurate except for C16:1ω9 and C22:6ω3. This indicates that the model based on desired properties only is not sufficient to explain the observed changes for all FAs. To further refine the model, we included LCPUFA synthesis and oxidation of some FAs.

– Elongation produces C22:6ω3 from C18:3ω3 and C20:4ω6 from C18:2ω6, without a need to consider intermediates explicitly. LCPUFA synthesis is composed of Δ5 and Δ6 desaturation, elongation and peroxisomal β-oxidation. The first step, desaturation of both C18:3ω3 and C18:2ω6 by D6D, is considered as limiting. Because substrates accumulates, we suppose that

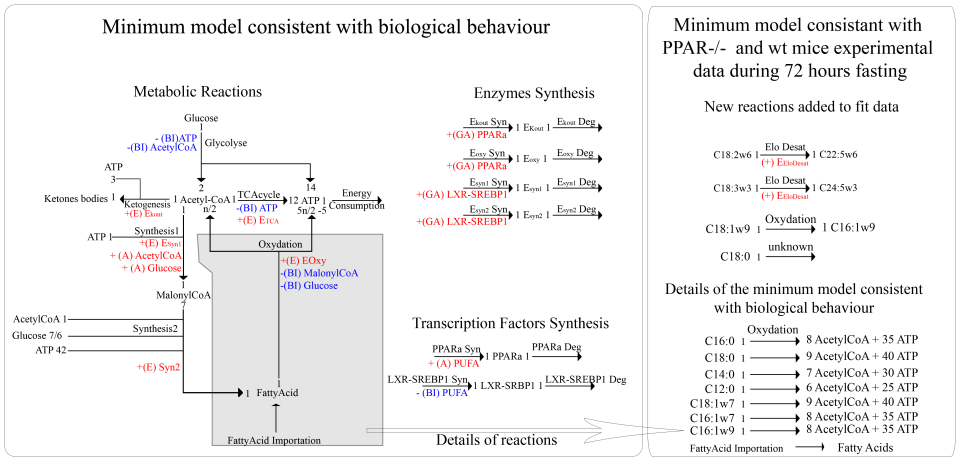

Figure 1. A reduced representation of lipid metabolism in mouse liver and its regulations. Each node stands for a product. Each arrow stands for a reaction combined with its stoechiometry. Reactions are regulated by the following processes: +(E) Enzymes catalyze, +(A) Activator, +(GA) Activating transcription factor, -(BI) Blocking inhibition, -(CI) Competitive inhibition.

the reaction is limited by the enzyme quantity and used the following formula: $Syn_{C22:6\omega3} = \frac{V_{syn}A_{C18:3\omega3}C18:3\omega3}{A_{C18:3\omega3}C18:3\omega3+A_{C18:2\omega6}C18:2\omega6}$ and $Syn_{C20:4\omega6} = \frac{V_{syn}A_{C18:2\omega6}C18:2\omega6}{A_{C18:3\omega3}C18:3\omega3+A_{C18:2\omega6}C18:2\omega6}$.

- We added oxidation and one of its intermediates reaction : C18:1ω9 produces C16:1ω9, other FAs are considered as fully oxidized. The activity of β-oxidation depends on the length of the FA: in PPAR-/- mice, being greater for shorter chain length. For each F in (C18:1ω9 : C18:1ω7, C18:0, C16:1ω9, C16:0), and assuming that the affinities are the same for these FAs, we set $Oxi_F = \frac{V_{oxi}F}{V_{oxi}+F}$ For the other FAs, we assumed $Oxi_F = 0$.
- The full model then becomes $\frac{dLiverF}{dt} = Imp_F + Syn_F + Oxi_F$ for each F.

From the biological data, we adjusted the values of K_{syn}, AC18:2ω6, AC18:3ω3 and V_{oxi} with a good score. Notice that ω3 LCPUFA synthesis is faster than ω6 synthesis, which is consistent with the literature data. The low value of V_{oxi} is consistent with the hypothesis of a low oxidation : 0.09 mmol/h of FA are accumulated while 0.003 are oxidated.

The model parameters were then adjusted with wild-type mice data, by assuming that the import ratio K_{imp} is still valid. Under this hypothesis, the sum of FAs fits with a constant oxidation rate of 0.062 mmol/h. As expected, the wild-type mice oxidize much more than PPAR -/-.

Model	Import	Elongation	Oxidation	Bad fitting score
Importation model in PPAR-/-	$K_{imp} = 0.22$	0	0	C16:1w9, C22:6w3
Importation, synthesis, short FA oxidation in PPAR-/-	$K_{imp} = 0.22$	$V_{syn} = 8\,10^{-4} mmol/h$ $A_{C18:3w3} = 0.98$ $A_{C18:2w6} = 0.02$	$V_{oxi} = 0.003 mmol/h$ for short FA $V_{oxi} = 0$ either	All good

4 Discussion

We proposed a modelling strategy to exhibit the smallest set of reduced metabolic reactions needed to explain a compendium of required properties together with experimental data. We applied our method to modelling fatty acid metabolism during a fed-to-fasting transition. Starting from a simple

model of FA metabolism, the modelling approach indicated that a small set of reactions was sufficient to properly fit the model to both mutant and wild-type fatty acid data.

We conclude that a minimal model in fasted PPAR-/- mice must include synthesis of C22:6ω3 from C18:3ω3. This reaction is known to be limited by D6D, whose expression is activated by both PPARα and SREBP1 [8,9]. However, PPARα is not present in PPAR-/- and SREBP1 activity decreases during fasting. This suggests that C22:6ω3 production in PPAR-/- mice should decrease but our analysis pointed out a constant accumulation rate. This indicates the need to measure D6D activity in both PPAR-/- and wild-type mice in order to check the prediction of the model. Confirmation would represent a strong indication for regulators of D6D that remain to be identified.

Our analysis also indicated a difference in oxidation rate between mutant and wild-type mice. This confirms the strong effect of PPARα on FA oxidation. Since the model fits with an oxidation rate that is dependant on the genotype and independent from nutritional status, we conclude that the nutritional regulation of oxidation mediated by PPARα is smaller than its constitutive effect.

In the future, we intend to expand the model by including PCR-experimental data on enzymes of FA oxidation and LCPUFA synthesis. Other experimental protocols will then be studied, such as fasting-to-fed transition and the reaction to different diets. The model will then be used to analyze the difference between mice and other species (like chicken) for which the liver is the main tissue for fatty acid synthesis.

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